Occurrence of arbuscular mycorrhizal fungi in the roots of two grapevine cultivars in response to bioproducts

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INTRODUCTION

Fruits of the cultivars ‘Regent’ (‘Diana’ × ‘Chambourcin’) and ‘Solaris’ (‘Merzing’ × ‘Geisenheim 6493’ (‘Zarya Severa’ × ‘Muscat Ottone’)) are mainly used for wine production. Both cultivars were bred in Germany and are classified as Vitis vinifera (L.) (Vitis International Variety Catalogue 2015) or as inter-intraspecific hybrids (Theocharis et al., 2010). ‘Regent’ and ‘Solaris’ have higher resistance to frost than most of the original V. vinifera varieties and have low susceptibility to fungal diseases (Lott et al. 2010; Lisek, 2012).

Both in Poland and abroad, bioproducts are increasingly being used in sustainable crop production (Sas Paszt et al., 2015). Agricultural bioproducts include diverse formulations of compounds, substances and other products that are applied to plants or soils to regulate and enhance the crop’s physiological processes, thus making them more efficient. Microbial bioproducts act on plant physiology through pathways different from those of nutrients to improve crop vigour, yield, quality and post-harvest shelf storability (Verplancken, 2011). Bioproducts affect plants by exerting favourable effects on plant primary and secondary metabolism (Hause and Fester 2005; Jung et al. 2012). They stimulate the synthesis of natural hormones, facilitate the uptake of minerals from the soil, improve root growth, and also contribute to increasing the resistance to adverse environmental conditions (e.g. drought, frost and cold) (Sharma et al., 2014; Battacharyya et al., 2015; Van Oosten et al., 2017). The requirements to protect the natural environment and human health steer the agricultural production towards production by organic and environmentally friendly means. This leads to an increasing use of bioproducts, natural plant protection products and soil conditioners. Proper use of natural plant protection products and fertilizers in organic crop production is one of the key elements of their effectiveness.

Arbuscular mycorrhizal fungi (AMF) live in symbiosis with the majority of plant species on Earth (Öpik et al., 2006; Shahabivand et al., 2016). In many studies, AM fungi have been observed to have a positive effect on the growth and development of grapevines. For example, symbiotic fungi are involved in the uptake and transport of nutrient elements by the host plant (Cheng & Baumgartner, 2006), and also increase water use efficiency (Valentine et al., 2006) and yielding of grapevines (Schreiner et al., 2007).

The numerous studies on grapevines have not examined the impact of bioproducts on the colonization of grapevine roots by arbuscular mycorrhizal fungi in field conditions. The aim of this study was to determine the effect of bioproducts on the colonization of grapevine roots by arbuscular mycorrhizal fungi under plantation conditions to develop sustainable wine-growing methods with the use of bioproducts of natural origin.

MATERIALS AND METHODS

Plants of ‘Solaris’ and ‘Regent’ grapevine cultivars, grafted on SO4 rootstock (V. berlandieri x V. riparia), were planted in the spring of 2008 in the Experimental Orchard of the Research Institute of Horticulture in Skierniewice (central
Physiological Response of Grapevine Cultivars to Mycorrhizal Fungi

The study on the colonization of grapevine roots by arbuscular mycorrhizal fungi conducted in 2009-2014 indicates favourable effects of the bioproducts used on increasing mycorrhizal frequency in the roots of the cultivars tested (Table 1). In the first years of the experiment (2009-2011), the highest mycorrhizal frequency in the cultivar ‘Solaris’ was observed in the roots of plants treated with the bioproducts BF Ekomix and Ausma applied separately or together with NPK fertilization. In the final years of the experiment (2013-2014), the bioproduct Biolisa was observed to have a positive effect on mycorrhizal frequency in plant roots. For the cultivar ‘Regent’, the most beneficial effect on mycorrhizal frequency in individual years of the experiment was exerted by the following bioproducts: BF Ekomix, manure, Ausma and Biolisa, applied on their own

### RESULTS AND DISCUSSION

The degree of colonization of grapevine roots by AM fungi was determined using the method of Trouvelot et al. (1986) with the use of a MYCOCALC program (http://www2.dijon.inra.fr/mychintec/Mycocalc-prg/download.html). The results were statistically analyzed using three-factorial analysis of variance with Tukey’s test at a significance level of α = 0.05 using STATISTICA v.10 software (StatSoft Inc., 2011). The only AM fungi determined in this study were those naturally occurring in the grapevine roots, because all the applied bioproducts did not contain any introduced AM fungi.

### TABLE 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>2009</th>
<th>2010</th>
<th>2011</th>
<th>2012</th>
<th>2013</th>
<th>2014</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solaris Regent</td>
<td>3.33 ab</td>
<td>4.44 cd</td>
<td>4.44 cd</td>
<td>4.44 cd</td>
<td>4.44 cd</td>
<td>4.44 cd</td>
</tr>
<tr>
<td>Solaris NPK</td>
<td>14.44 fg</td>
<td>11.0 ed</td>
<td>11.11 d</td>
<td>11.11 d</td>
<td>11.11 d</td>
<td>11.11 d</td>
</tr>
<tr>
<td>Solaris NPK + Ausma</td>
<td>16.67 gh</td>
<td>11.11 d</td>
<td>11.11 d</td>
<td>11.11 d</td>
<td>11.11 d</td>
<td>11.11 d</td>
</tr>
<tr>
<td>Solaris NPK + Bioilsa</td>
<td>17.78 h</td>
<td>12.22 e</td>
<td>12.22 e</td>
<td>12.22 e</td>
<td>12.22 e</td>
<td>12.22 e</td>
</tr>
<tr>
<td>Solaris NPK + Manure</td>
<td>22.22 g</td>
<td>17.78 h</td>
<td>17.78 h</td>
<td>17.78 h</td>
<td>17.78 h</td>
<td>17.78 h</td>
</tr>
<tr>
<td>Solaris BF Ekomix</td>
<td>33.33 i</td>
<td>33.33 i</td>
<td>33.33 i</td>
<td>33.33 i</td>
<td>33.33 i</td>
<td>33.33 i</td>
</tr>
<tr>
<td>Solaris BF Ekomix + NPK</td>
<td>33.33 i</td>
<td>33.33 i</td>
<td>33.33 i</td>
<td>33.33 i</td>
<td>33.33 i</td>
<td>33.33 i</td>
</tr>
</tbody>
</table>

Averages marked by the same letter do not differ significantly at the p = 0.05 according to Tukey’s multiple test. The averages were compared for two cultivars, separately for every year.

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(without additional NPK fertilization). In contrast to the years 2009-2011 and 2013-2014, the highest mycorrhizal frequency recorded in 2012 was in the roots of plants treated with Bioilsa combined with NPK fertilization.

Compared with NPK treatment, the applied bioproducts and bioproducts combined with NPK fertilization contributed to increasing the colonization of roots by AM fungi. NPK fertilization limited to the greatest extent the degree of mycorrhizal association in the roots of ‘Solaris’ (11.7%) and ‘Regent’ (7.4%). The results for the six years of investigation show that the highest mycorrhizal frequency was recorded in the roots of ‘Solaris’ grapevines after applying the bioproduct Ausma (28.2%). For ‘Regent’, the highest mycorrhizal colonization frequency was observed in the plants treated with the bioproducts BF Ekomix (30.0%), Ausma (27.9%), and manure (29.8%). The combined application of the bioproducts with NPK fertilization resulted in the lowering of the degree of mycorrhizal association in the roots of the grapevines in comparison with the use of the bioproducts alone. The data revealed that numerous vesicles in the roots of ‘Solaris’ and ‘Regent’ grapevine cultivars were formed after the application of the bioproduct Bioilsa, whereas single vesicles were detected in the roots of the plants under standard NPK fertilization (Figure 1, 2). Although the NPK treatment reduced mycorrhizal frequency in grapevine roots, the mycorrhizal frequencies for NPK and the control without application of bioproducts did not differ significantly. The NPK treatment also did not differ significantly with regard to mycorrhizal frequency after application of the bioproducts except for Ausma on ‘Regent’ and ‘Solaris’ and BF Ekomix on ‘Regent’.

In this study, the species of arbuscular mycorrhizal fungi were identified in the rhizosphere of cultivars ‘Regent’ and ‘Solaris’ based on the morphological traits of their spores: *Funneliformis caledonium*, *Glomus aggregatum*, *Glomus macrocarpum*, *Rhizophagus intraradices*, *Claroideoglomus claroideum* and *Gigaspora margarita* (data not presented). The results from the individual years of the study and 6-year averages confirmed the negative impact of NPK fertilization in the mycorrhizal frequency in the roots of both grapevine cultivars tested. The use of bioproducts, such as Ausma, Bioilsa, BF Ekomix and manure, seems to partly reverse the negative effect of inorganic fertilization on the colonization of roots by mycorrhizal fungi, though the differences between mycorrhizal frequency (F%) in the roots after NPK fertilization and NPK fertilization with bioproducts were not statistically significant. Mycorrhizal frequency in the roots of the grapevines was low at the average level. Low mycorrhizal frequency has also been observed in the roots of apple (2.5% to 25.8%) and blackcurrant plants (0 to 11.1%) treated with organic mulches (Sumorok et al., 2011; Derkowska et al., 2013). Petit and Gubler (2006) recorded higher colonization of roots at a level of 48.3% in the plants of *Vitis rupestris* inoculated with *Rhizopagus intraradices*, and at 54.5% in the plants inoculated with *Rhizopagus intraradices* and the pathogenic fungus *Cylindrocarpon macrodidymum*. A factor that is of significance to mycorrhizal frequency in the roots of grapevines is soil moisture. The water content of the soil, weather conditions, frequency of irrigation or its absence all have a significant impact on mycorrhizal frequency in the roots of grapevine plants (Augé, 2004; Schreiner et al. 2007).

Mycorrhizal frequency in the roots of plants is also significantly affected by soil fertility in terms of mineral and organic matter content, and by soil pH. The lowest frequency was observed in the combination fertilized with NPK, and it might have been influenced by the form of the nitrogen fertilizer used, containing both nitrate and ammonium ions, which more strongly limit the colonization of roots by AMF. Reductions in mycorrhizal frequency under the influence of ammonium ions have been observed by other authors (Guo et al., 2006; Karagiannidis et al., 2007). It has also been found that the presence of phosphate in the soil or application of phosphorous fertilizers reduces AMF frequency/colonization in plant roots. Frequency of occurrence, relative abundance, species richness and diversity index of AM fungi were higher in low-P soils than in high-P soils (Harikumar and Potty 2012).

Moreover, mycorrhizal frequency is also affected by the pH of the soil – it is lower at a lower pH (Wang et al., 1993). Application of the bioproduct Bioilsa increased the number of vesicles in the roots of both grapevine cultivars tested compared to the plants under standard NPK fertilization. The use of manure and bioproducts, such as: Ausma, Bioilsa and BF Ekomix, seems to reverse the negative effect of mineral fertilization on the colonization of roots by mycorrhizal

![FIGURE 1](image1.png)

Numerous vesicles in the roots of a ‘Regent’ grapevine treated with bioproduct Bioilsa

![FIGURE 2](image2.png)

Single vesicles in the roots of a ‘Regent’ grapevine fertilized with NPK
fungi. Research in this area will be continued in order to determine the impact of bioproducts on the occurrence of beneficial groups of microorganisms in the microbiome of the grapevine cultivars studied and on the growth and yielding of plants. This will help to develop sustainable wine-growing methods with the use of bioproducts of natural origin.

CONCLUSIONS
Application of the bioproduct Bioilsa resulted in the formation of numerous vesicles in the roots of ‘Solaris’ and ‘Regent’ grapevine cultivars. Colonization of grapevine roots by mycorrhizal arbuscular fungi was decreased to the greatest extent by mineral NPK fertilization. The use of manure and bioproducts, such as: Ausma, Bioilsa, BF Ekomix seems to reverse the negative effect of mineral fertilization on the colonization of roots by mycorrhizal fungi.

LITERATURE CITED


